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Mutagenicity of Indoor Air Particles in a Residential Pilot Field Study: Application and Evaluation of New Methodologies

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THE MUTAGENICITY OF INDOOR AIR PARTICLES IN A RESIDENTIAL PILOT FIELD STUDY: APPLICATION AND EVALUATION OF NEW METHODOLOGIES

JOELLEN LEWTAS*†, SUMIO GOTO‡, KATHERINE WILLIAMS†, JANE C. CHUANG§, BRUCE A. PETERSEN¶ and NANCY K. WILSON†

†Health Effects Research Laboratory and Environmental Monitoring Systems Laboratory, Environmental Research Center, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, U.S.A., ‡Department of Community Environmental Sciences, The Institute of Public Health, 6-1, Shirokanedai 4 Chome, Mineto-Ku, Tokyo 108, Japan, §Battelle Columbus Division Columbus, OH 43201, U.S.A. and ¶Applied Technology and Mobile Environmental Analysis, Sciex, Inc., 100 East Wilson Bridge Road, Suite 208, Worthington, OH 43085, U.S.A.

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Abstract—The mutagenicity of indoor air particulate matter has been measured in a pilot field study of homes in Columbus, Ohio during the 1984 winter. The study was conducted in eight all natural-gas homes and two all electric homes. Particulate matter and semi-volatile organic compounds were collected indoors using a medium volume sampler. A micro-forward mutation bioassay employing *Salmonella typhimurium* strain TM 677 was used to quantify the mutagenicity in solvent extracts of microgram quantities of indoor air particles. The mutagenicity was quantified in terms of both mutation frequency per mg of organic matter extracted and per cubic meter of air sampled. The combustion source variables explored in this study included woodburning in fireplaces and cigarette smoking. Homes in which cigarette smoking occurred had the highest concentrations of mutagenicity per cubic meter of air. The average indoor air mutagenicity per cubic meter was highly correlated with the number of cigarettes smoked. When the separate sampling periods in each room were compared, the mutagenicity in the kitchen samples was the most highly correlated with the number of cigarettes smoked.

Key word index: Indoor air, mutagenicity, cigarette smoke, fireplaces, wood-burning, gas heat, gas stoves.

INTRODUCTION

Human exposure to mutagens and potential carcinogens in indoor air is of concern since we spend approximately 80% of our time indoors (National Academy of Sciences, 1981; Szalai, 1972), either in our homes, workplaces or schools. Studies in an office building (Lofroth *et al.*, 1983) and a cabin (Alfheim and Ramdahl, 1984) have shown that tobacco smoke can increase the mutagenicity of the indoor air substantially. These investigators used the *Salmonella typhimurium* plate incorporation assay (Ames *et al.*, 1975) to measure mutagenicity. They have reported that it is often not possible to measure the mutagenicity of indoor air when there is no smoking due to the low concentrations of particles and relatively large sample size required by the standard Ames assay. Subsequent studies in our laboratories (Morin, 1985) have confirmed this finding.

To measure the mutagenicity of indoor air in the absence and presence of tobacco smoke and to determine the effect of other combustion sources (such as gas stoves and fireplaces), we have used a micro-forward mutation assay (Skopek *et al.*, 1978a, b; Thilley *et al.*, 1983), which was modified in our laboratories (Goto *et al.*, in preparation). This assay utilizes an isolate of *Salmonella typhimurium* TM 677 and requires approximately one-tenth the sample size of the Ames assay.

This paper reports the mutagenicity results of a pilot field study of ten homes in which this micro-forward mutation assay was used to bioassay the particulate organic matter from three rooms in each home. Other aspects of this study, including chemical analysis of polycyclic aromatic hydrocarbons (PAH), are reported elsewhere (Wilson *et al.*, 1985). The sources examined in the study were natural gas heat and appliances, fireplaces and cigarette smoking.

MATERIALS AND METHODS

Pilot study design

Eight study homes and two background homes were selected from the Columbus, Ohio area for sampling in the winter (March 1984). The study included homes with and without cigarette smoking occupants, with and without wood-burning fireplaces in use; all used gas heat and appliances. Background homes were all-electric homes with no smokers and no fireplaces. Table 1 lists the matrix of home and sample characteristics selected as stratification variables in this study. Two of the samples, C2 and F2, were obtained as repeat collections at homes C and F.

* Author to whom correspondence should be addressed.

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Table 1. Matrix of home and sample characteristics

Utilities	Fireplace	Smoking	Code	Air exchange rate (h^{-1})	House volume (ft^3)	Volume sampled* (%)
Electric	–	–	G	0.67	13,500	5.1
Electric	–	–	H	0.69	14,100	4.8
Gas	–	–	A	0.34	12,000	12.4
Gas	–	–	I	0.37	—	—
Gas	+	–	B	0.35	14,200	8.2
Gas	+	–	C	—†	14,200	11.2
Gas	+	–	C2	0.29	14,200	10.4
Gas	–	+(12)‡	E	0.21	6500	34.3
Gas	–	+(58)	J	0.41	13,400	8.4
Gas	+	+(36)	D	0.21	12,400	17.6
Gas	+	+(13)	F	0.74	13,000	8.0
Gas	+	+(16)	F2	0.32	13,000	7.8

* Sampling volume as a percentage of the total air volume through the house in 24 h.
† Sample was lost.
‡ Number of cigarettes smoked in the 24-h period.

The objectives of the pilot field study included the evaluation of analysis methodology for determining the concentration of polynuclear aromatic hydrocarbons (PAHs) and nitro-substituted PAHs (NO_2–PAHs) in residential air. These results are reported elsewhere (Wilson *et al.*, 1985). The samples collected and prepared for chemical analysis were divided. One half of the sample was used for PAH and NO_2–PAH analysis and one half was prepared for the microbial mutagenesis studies reported here. The bioassay studies were designed to determine the mutagenicity of residential air and estimate the influence of smoking, wood-burning fireplaces, and gas utilities on the concentration of mutagenic activity per cubic meter of air.

Sampling and preparation of samples for bioassay

A modified EPA medium volume air sampler with a rotary carbon vane oil-less vacuum pump was used in the indoor air sampling; a modified EPA medium volume sampler with a bypass motor (General Metal Works, Inc.) was used in the outdoor air sampling. The sampler was equipped with a quartz fiber filter followed by a polyurethane foam adsorbent cartridge. The sampling head was mounted on a tripod to place the inlet 60 in. (152 cm) from the floor. A heavy wall 1 in. (2.54 cm) Tygon tube led through a window port from the sampling head to the pump, which was placed in a sound-insulated box just outside the house. Indoor samples were collected over consecutive 8-h periods, at times and in locations chosen to correspond with the occupants' activities. The sampling times in the kitchen were from 0700 to 1500 h, in the living room from 1500 to 2300 h and in the bedroom from 2300 to 0700 h. The sampler flow rate was approximately 12.5 $m^3\ h^{-1}$, which collected 100 m^3 of air over 8 h. During the entire indoor sampling, a single outdoor air sample (24-h, 300 m^3) was collected in the back yard of each study home. Air exchange data were obtained by measuring the decay of SF_6 injected at the start of the sampling period, followed by sampling at 1, 3, 5, 7 and 8 h with air-tight syringes and subsequent analysis by gas chromatography (electron capture detector). The room and house volumes were estimated from the dimensions of the rooms in each home. Questionnaire/diaries were used to record the number of cigarettes smoked and other activities that might affect the PAH concentrations during the sampling period.

Preparation of samples for bioassay

The filters were placed in a Soxhlet extractor and extracted with methylene chloride for 16 h. The solvent extracts were concentrated by Kuderna–Danish evaporation without taking the samples to dryness. An aliquot was removed from each extract and used for gravimetric mass determination. The samples were divided in half: one half of the sample was used for chemical analysis of the PAHs, NO_2–PAHs and several other marker compounds (Wilson *et al.*, 1985). The other half was concentrated to an appropriate volume and transferred into 1 dram glass screwtop vials to obtain the following doses: 0.4, 0.2, 0.05 and 0.02 mg per vial. Dimethyl sulfoxide (DMSO, 0.002 ml) was added to each vial and the methylene chloride evaporated under a gentle stream of dry nitrogen. These samples were shipped on dry ice and stored at −80°C prior to bioassay, which was performed in the same vials.

Mutagenesis bioassay

The micro-forward mutation assay (Fig. 1) was conducted according to the procedure of Goto *et al.* (in preparation) using *Salmonella typhimurium* strain TM 677 which was originally obtained from Dr W. G. Thilly, at the Massachusetts Institute of Technology, and reisolated (Goto *et al.*, in preparation). The bacterial mixture (0.098 ml) containing TM 677 and the metabolic activation system, when used, was added directly to the sample vial to give a total volume of 0.100 ml. The vials were incubated at 37°C in a rotating tissue culture drum (Lab-line) for 60 min. When the metabolic activation system was used, it was added to the bacterial mixture as 10% v/v S9 prepared from male Charles River CD-1 rats induced with Aroclor-1254 (Ames *et al.*, 1975); 10% cofactors [prepared from G6P (10 mg ml^{-1}), NADP (10 mg ml^{-1}) and $MgCl_2$ (6.7 mg ml^{-1})]; 1% G6PDH (40 units ml^{-1} of 5 mM Na citrate); and 79% of the bacteria dilution (1/10). Triplicate plates were used to determine both the 8-AG resistant (mutant) colonies and the surviving colonies. Colonies were counted on an Artek Model 880 colony counter. The mutation frequency was calculated from the resistant colonies and the surviving colonies as described below.

Benzo(*a*)pyrene (BaP) and 9-aminoacridine (9-AA) were used for the positive control chemicals. 8-Azaguanine (8-AG), glucose-6-phosphate (G6P), NADP monosodium salt (NADP) and glucose-6-phosphate dehydrogenase Type XV (G6PDH) were obtained from Sigma Chemical Co. (St. Louis, MO).

Range-finding experiments were first run with and without metabolic activation at doses from 5 to 1000 $\mu g\ vial^{-1}$ using samples from the kitchens and living rooms of three homes (D, E and J). These three homes had cigarette smoking and were gas-heated, either with or without an operating fireplace

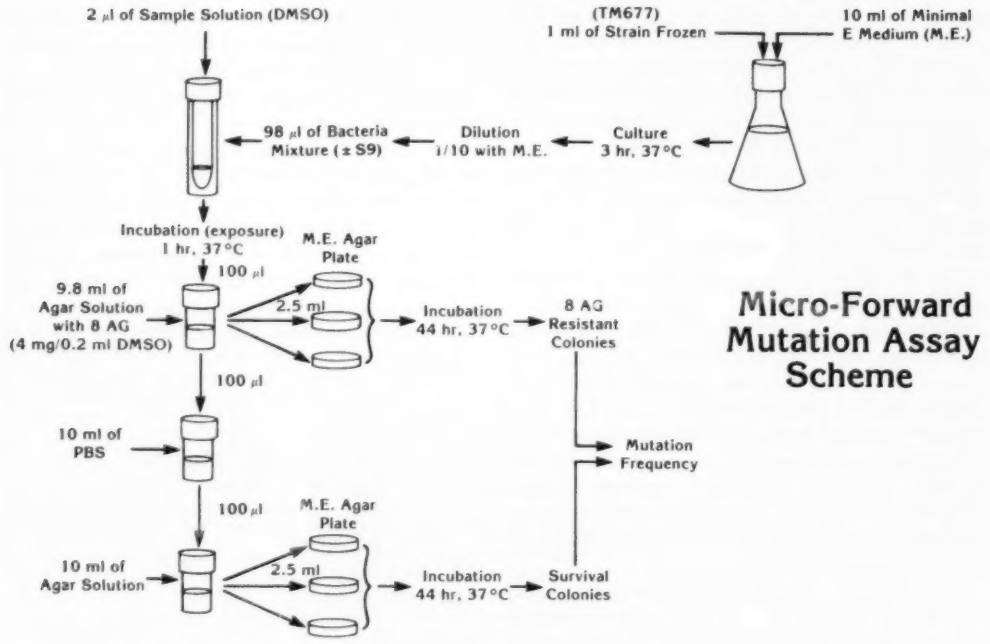

Fig. 1. Micro-forward mutation assay scheme.

(see Table 1). The organic mass extracted from samples collected from these homes was sufficient to allow a comparison of the mutagenicity of these samples in the micro-forward assay and in the Ames plate incorporation, reverse mutation assay. Samples from the kitchens of homes D and J and the living rooms of homes E and J were run in the Ames assay. The Ames assay was conducted according to the procedure of Ames and coworkers with minor modifications by Claxton (Ames *et al.*, 1975; Claxton, 1981) using strain TA 98 with and without metabolic activation at doses from 5 to 1000 μg per plate.

Based on the above range-finding studies, four doses (20, 50, 200 and 400 μg) were selected for the larger comparative experiments. In these experiments, the samples from the same room in all houses (either the kitchen, living room or bedroom) or outdoors were assayed at one time in one experiment at 20 and 50 μg and in a second experiment at 200 and 400 μg, except where insufficient mass was available for all doses. In these comparative experiments, all samples were assayed in the presence of metabolic activation.

The data analysis utilized only the data from 50, 200 and 400 μg doses from the comparative experiments since the 20 μg dose did not generally result in a mutation frequency above the spontaneous (solvent control). The mutation frequency (MF) for each dose was calculated by dividing the average number of mutant colonies (three plates) by the average number of surviving colonies (three plates) and multiplying by the dilution factor (1×10^{-4}). The net mutation frequency (MF-spontaneous MF) per mg of extractable organics was determined and multiplied by the mg of extractable organics collected m^{-3} of air volume sampled to give the mutagenicity m^{-3} ($MF \times 10^{-5}\ m^{-3}$).

RESULTS AND DISCUSSION

The mass of particulate matter and methylene chloride-extractable organics (range and mean) for each of the variables across all rooms of the house are shown in Table 2. The concentration of particulate

Table 2. Mass of particulate matter and extractable organics collected across all rooms and outside

		Particulate matter (mg)		Extractable organics (mg)*		
Source parameters	No. Homes	(Range)	(Ave.)	(Range)	(Ave.)	(%)
Electric	2	1.3–6.0	2.9	0.4–1.6	1.1	38
Gas	2	2.2–38.7†	10.1	1.0–25.8	5.4	53
Gas/smoking	2	5.5–33.2†	15.6	3.1–28.9	14.4	92
Gas/fireplace	2	2.7–9.2	4.3	0.7–4.4	1.7	40
Gas/smoking/fireplace	2	4.7–20.8†	10.2	1.6–18.6	5.2	51
Outside‡		3.9–22.6	15.9	0.4–8.7	2.8	18

* Half of this mass was available for bioassay studies.

† Highest values in kitchens.

‡ 300 m^3 of air was sampled outside over 24 h while 100 m^3 was sampled in each room inside over 8-h periods.

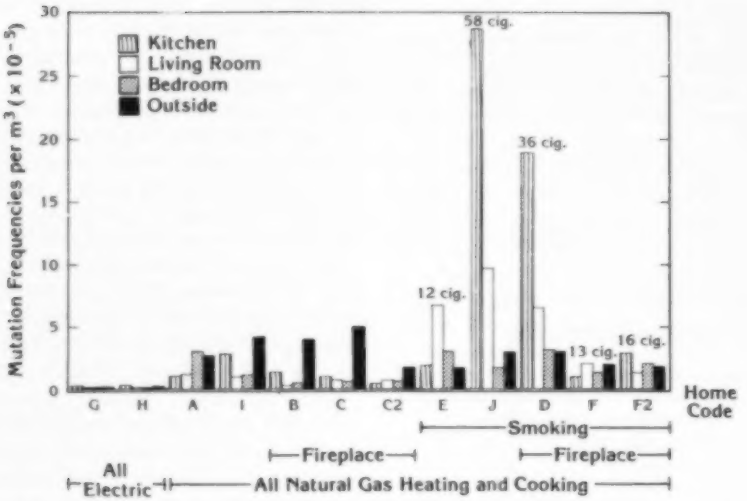

Fig. 4. Mutagenicity per volume of air sampled in each room and home in the field study.

Table 6. Correlation matrix of mutagenic activity between rooms and with number of cigarettes smoked

	Living room	Sleeping area	Outdoor	Average of indoor	Cigarette
Kitchen	0.8465	0.3748	0.3122	0.9817	0.9553
Living room	—	0.6444	0.2466	0.9281	0.8986
Sleeping area	—	—	0.2345	0.5186	0.4592
Outdoor	—	—	—	0.3115	0.2273
Average of indoor	—	—	—	—	0.9682

99.9% Confidence value: 0.8233.
99% Confidence value: 0.7079.
95% Confidence value: 0.5760.

show good correlations with the indoor data (Table 6). The outdoor mutagenicity was very similar for the eight homes with gas utilities (1.6–3.9 MF $\times 10^{-5}$ m^{-3}). The two electric homes were newer and were located in a neighborhood of all-electric houses. The outside mutagenicity was significantly less (approximately 1/10) than outside the gas homes which could be due in part to weather conditions at the time of sampling. The inside mutagenicity across all the rooms in the two electric homes was also significantly less than all of the inside mutagenicity for the gas homes. Since this study included only two electric homes with none of the combustion source variables of the gas homes, we do not have sufficient data to conclude that gas homes have a higher concentration of particulate mutagenicity than electric homes. Future studies, however, will be planned to examine this possibility.

Although we observed a trend toward higher mutagenicities in the kitchens compared to the living rooms and bedrooms; in the absence of cigarette smoking, the differences were less. In general, the homes in which there was no smoking had concentrations of particle mutagenic activity inside which were lower than outside. A significant amount of semi-volatile organic matter was collected on the PUF cartridges after the particle filters. These organic compounds are also mutagenic and studies are in progress to quantitate their contribution to the total mutagenicity in these homes.

In summary, we found that the concentration of particle associated mutagenicity per cubic volume of air was the greatest in homes with cigarette smoking and was highly correlated to the number of cigarettes smoked. The micro-mutagenesis method employed in this study also provided quantitative data on the mutagenicity of the extractable particulate organics from all the homes in this study including the non-smoking homes both with and without gas utilities and fireplaces. The results from these non-smoking homes suggest that further studies are needed to examine both cooking and gas utilities as sources of mutagenic

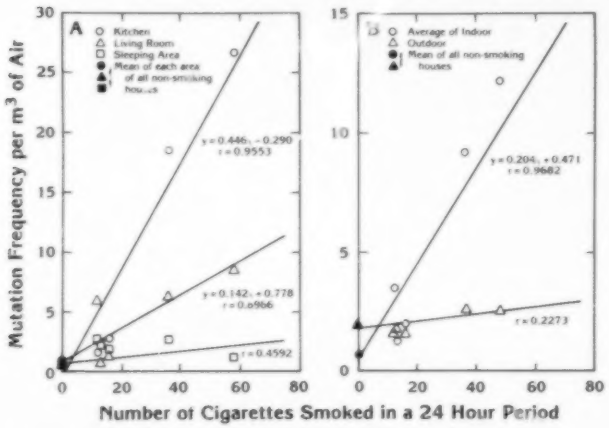

Fig. 5. Correlation of mutagenic activity (mutation frequency $\times 10^{-5}$ m^{-3}) with the number of cigarettes smoked in each home. Plot A shows the correlation for individual rooms: kitchen, living room and bedroom for smoking homes with the number of cigarettes. Plot B shows the correlation for both the outdoor samples and the average of all the indoor rooms with the number of cigarettes. The mean of each area for all non-smoking homes is also presented.

organics inside the home. Furthermore, the influence of outdoor sources as contributors to the mutagenicity indoors needs to be investigated.

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